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Acquired Immunity to Pathogenic Fungi in Gnotobiotic Animals

Final Comprehensive Report

(July 1974 - February 1978)

Edward Balish, Ph.D.

February 1979

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Fungal infections were studied in germfree and conventional guinea pigs, rats and mice. The response of flora-defined and conventional nude (congenitally athymic) mice to pathogenic fungi was also investigated.

In brief dermatophyte skin infections on conventional guinea pigs were of much shorter duration (2½ times) than comparable infections on germfree guinea pigs. Dermatophyte infections on the skin of guinea pigs were associated with an impairment (suppression) of in vitro lymphocyte blastogenesis to mitogens and

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dermatophyte antigens. The suppressor, also present in infected gnotobiotic guinea pigs appeared to be a serum component that disappeared from serum when the fungal load on the skin was diminished.

Germfree and conventional rats did not undergo chronic skin infections when challenged with dermatophytes that produced full-blown infections in guinea pigs. The dermatophytes did not colonize the gastrointestinal tract of germfree rats or guinea pigs.

Congenitally athymic mice (nude) also were not cutaneously infected when challenged with T. mentagrophytes mycelia or spores.

Scanning electron microscopy (SEM), light microscopy, and quantitative culture of microorganisms in intestinal contents were used to determine the effects of oral tetracycline, the bacterial flora and thymus-dependent immune compentency on the capacity of Candida albicans to colonize and infect the gastrointestinal tract of four groups of mice: (a) thymus-intact conventional mice, (b) conventional athymic mice, (c) flora-defined athymic mice and (d) thymus-intact bacteria-free mice.

Thymus-intact conventional mice without antibiotic treatment began to shed C. albicans less than 48 hours after oral yeast challenge, and were devoid of detectable yeasts after 16 days. Tetracycline, however, altered the bacterial flora qualitatively and quantitatively, and allowed C. albicans to colonize in less than 48 hours and to persist in the gut tract for 32 days. Only 2/72 conventional mice developed candidiasis (hyphal infection).

Although tetracycline altered the bacterial flora of conventional athymic (nude) mice, it was not required to allow C. albicans to colonize their gut tract to levels significantly higher than in thymus-intact conventional mice. Although all nude mice were consistently colonized and 14/24 animals showed an increased yeast colonization of the keratinized stomach, only 3/24 developed gastric candidiasis. Flora-defined athymic mice had significantly lower aerobic bacterial levels and significantly higher C. albicans levels in the gut contents than conventional athymic mice. The flora-defined mice, however, developed gastric candidiasis by day 5.

Bacteria-free mice were uniformly colonized and infected with C. albicans less than 48 hours after oral challenge regardless of tetracycline treatment. Populations of C. albicans in the gut of bacteria-free mice were significantly higher than in the gut tract of the conventional or athymic mice. Gastric mycelial infection was detected in 8/10 of the gnotobiotic animals 2 days after oral challenge. By 32 days, 45/50 of both tetracycline treated and control gnotobiotic mouse groups were infected with C. albicans.

These data indicate that a competitive bacterial flora is more effective in preventing C. albicans infection than an intact immune system, and that an immune deficiency may allow increased yeast colonization of the keratinized and glandular stomach epithelium. Tetracycline did not appear to enhance the invasiveness or pathogenicity of C. albicans in mice even though it facilitates yeast-phase gut colonization in conventional mice.

Acquired Immunity to Pathogenic Fungi in Gnotobiotic Animals

Final Comprehensive Report

Contract No. DAMD 17-75-C-5004

(July 1974 - February 1978)

By

Edward Balish, Ph.D.

Professor

Departments of Surgery and Medical Microbiology

TABLE OF CONTENTS

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INTRODUCTION

Germfree animals are a unique animal model for studies on infectivity, pathogenesis, acquired-immunity, prophylaxis, and therapy of dermatophyte infections because: 1) there are no skin or gut bacteria to augment (or hinder) the true course of an experimental dermatophyte infection, 2) germfree animals have not had any previous exposure to viable dermatophytes and thus the problem of contending with immunological effects from prior subclinical fungal infections is eliminated, 3) true immune responses (both antibody, AMI, and thymus-dependent cell-mediated immunity, CMI) can be assessed without competition and/or antigenic stimulation by the viable bacteria and other fungi that are so prevalent in, and on, experimental animals, 4) when activated, the antibody and cell-mediated immune responses of the germfree animal are every bit as good as, and in some instances better than, the conventional animal; therefore, an assessment of pure primary induction of CMI and AMI occurs, 5) therapy (either topical or systemic, i.e. steroids or Griseofulvin) on the dermatophyte infection can be assessed without interference from competing bacteria, or previous subclinical dermatophyte infections, 6) the effect of skin microorganisms or their inhibitory products, on the course of dermatophyte infections, can be evaluated in the gnotobiotic model, 7) cellular infiltrates of truely primary skin test responses can be assessed in gnotobiotic animals, and 8) germfree nude mice (athymic) can be used to study the early stages of dermatophyte growth and infectivity on the skin in a bacteria-free or defined-microbial environment.

It is now known that T. mentagrophytes and other dermatophytes, when looked for, can be isolated from all conventional guinea pig, rat, and mouse colonies. This limits the usefulness of conventional guinea pigs, rats, and mice in studies on immunity, prophylaxis and therapy of dermatophyte infections.

Our research program is using germfree rats, guinea pigs, and nude mice as models for dermatophyte infections. We have studied dermatophyte infections in conventional rats, guinea pigs and flora-defined nude mice (i.e. an animal without a functional T-cell capability). Our final results with each of these animal models will now be detailed.

Microorganisms: Our studies have been carried out with Trichophyton mentagrophytes; work on C. albicans, used in some of these studies is also supported by a grant from the Research Corporation (Minneapolis, Minn.) and by an NIH training grant (AI-0045-04).

General Comments:

Germfree and Conventional Guinea Pigs: Our infections of conventional guinea pigs (Hartley strain and strain 2) have manifested the gross pattern of dermatophyte infection already described by the LAIR group (Akers, Kerbs, Jones, et al.) for conventional guinea pigs. Our only information to add to their conventional system is in the area of the cellular infiltration (histology) of lesions and skin tests and on lymphocyte blastogenesis assays. We saw much more eosinophil and neutrophil involvement in lesions and skin tests (on conventional guinea pigs) than one would normally expect in a classical PPD delayed-type hypersensitivity response.

Randomly bred strain 2 guinea pigs (i.e., the conventional controls of germfree guinea pigs used in this study) were shaved (on the back) and inoculated with a 2-4 week old mycelial phase culture of T. mentagrophytes that was grown on Sabourauds' agar. No occlusive dressings were applied. The temporal course of infection is shown in Table I. Groups of animals were sacrificed at weekly intervals beginning with day 0 and squares of infected lesion (skin) were excised (3/animal) and ground (in glass tissue grinders) and plated out (10-fold dilutions) in order to enumerate colony forming units of T. mentagrophytes (CFU's). Irrespective of inoculating dose (10^2 or 10^4) our results showed that colony forming units peaked between days 7-14 and then decreased. This decrease in CFU's correlated with the gross resolution and clearance of the lesion. See Materials and Methods for methodology on skin CFU's.

Lymphocyte Transformation: Spleens and a pool of lymph nodes (cervical-tracheal, axillary and supra-scapular) were excised from the infected animals (three animals at each sacrifice). Tissues were minced and pushed through 60-mesh stainless-steel wire screens; washed 3X in PBS; resuspended in RPMI and diluted to give a concentration of 1-2 x 10^5 cells/well (microtiter plates) with 5% homologous (heated) guinea pig serum. Various concentrations of antigens (10-50 µg) and mitogen (1-50 µg) are added to lymphocytes on day 0. Cultures are then incubated for 96 hours at which time 2 µCi of ^{3}H-thymidine are added to each well of a microtiter plate. Lymphocytes from each well of the microtiter plate are precipitated on fiberglass filters and counted for radioactivity. Stimulation indices for each mitogen or antigen were calculated by dividing:

$$\frac{\text{cpm stimulated}}{\text{cpm controls}} = \text{S.I. stimulation index}$$

The capacity of splenocytes to respond to mitogens (ConA and PHA) decreased markedly over the first 14 days of the dermatophyte infection and then started to return to normal on day 21. No positive splenocyte blastogenesis was observed with T. mentagrophytes antigens during the first 21 days.

Contrary to splenocytes, a much improved response of lymph node lymphocytes was observed with ConA and PHA on day 21 and a response to dermatophyte antigen was observed at that same time. A suppressive effect on mitogen induced lymphocytes blastogenesis was observed, during the first 14 days after infection.

As the antigenic load of T. mentagrophytes on the skin increased, blastogenesis to polyclonal mitogens decreased; to a maximum of 90% on day 14. The blastogenesis began to increase and response to dermatophyte antigen was evident as the fungal load was diminished.

These are most interesting observations that have not been made previously on dermatophyte infections. A better understanding of this suppressive process and the mechanism of suppression could result in some basic new information on chronic dermatophyte infections. (see Progress Report 1977-1978 for further details).

Germfree Guinea Pigs: We have now for the first time infected germfree guinea pigs with T. mentagrophytes. The overall infection rate was 100%. The infection and host response was more severe and persistent (no regrowth of hair and still obvious, open, serous lesions at 50 days past infection in one of four guinea pigs). The infection did not spread beyond the original site of inoculation and no obvious fungal overgrowth occurred in the germfree isolator. There was no apparent fungal colonization of the nasal cavity, oral cavity or rectal area of the bacteria-free guinea pigs. We allowed the infected animals

(primary) to continue on in order to see how long it took for the lesion to clear and hair to grow back in the monoassociated (gnotobiotic) state. The hair grew back on all animals by 85 days. Almost 2½ times as long as comparable conventional controls. This is a true primary infection.

This germfree model could be an excellent one to study therapy and prophylaxis of fungal infections because of its chronic persistent nature. Also, it is very obvious that prior immunological experiences (the microbial flora) of conventional guinea pigs does reduce the severity and duration of dermatophyte infections in the conventional state. The lesions on conventional guinea pigs are not as severe or as prolonged as we have observed in the germfree guinea pig model.

To date we have seen that a secondary infection in the T. mentagrophytes gnotobiotic guinea pig runs a course very similar to the course observed in a primary infection of conventional animals. Thus, prior experience with a dermatophyte in the absence of other microorganisms does shorten the duration and severity of the secondary infection (acquired-immunity?). Monoassociated guinea pigs do respond to the polyclonal mitogen (PHA, ConA, Pokeweed mitogen) and to specific dermatophyte antigens that are injected intradermally. There is also a positive delayed hypersensitivity skin test (to purified T. mentagrophytes antigen) in the gnotobiotic guinea pigs that have cleared a primary infection. Histology of lesions, skin tests, and temporal fungal load and blastogenesis are similar to those described in conventional animals.

Studies with Germfree and Conventional Rats: Can dermatophytes, by themselves, infect rats that are free of a viable bacterial and fungal flora? To date our results indicate that germfree rats (and guinea pigs) can be infected by a pure culture of dermatophyte. Obvious infectious lesions, were observed in germfree and conventional rats, challenged with T. mentagrophytes. We were unable to cause any obvious dermatophyte lesions with Microsporum canis or with Epidermophyton floccosum in the germfree rats. It is also worth noting that none of the three fungi used i.e. E. floccosum, M. canis, or T. mentagrophytes was able to colonize the bacteria-free GI tract, oral cavity, nasal cavity or lungs of the germfree rats: only T. mentagrophytes was found in low numbers (10^2 - 10^4 gm of the feces from cecum and colon only). This is most interesting because even in the absence of competing bacteria, these fungi do not colonize or invade mucosal epithelial cells or keratinized stomach epithelium. Conversely, the germfree rat does show invasive hyphae in the keratinized portion of their stomach after monoassociation with C. albicans.

Another interesting aspect of the germfree rat model is that T. mentagrophytes infects (hyphae invade epidermis) the skin at the inoculated site. None of the germfree rats showed any lesions other than at the site of inoculation and only erythema was evident. No fungal overgrowth occurred in the chambers or on the animals skin at sites other than those experimentally infected. The T. mentagrophytes lesion (a red erythema) in germfree rats clears in 14 days. The lesion is not as severe as we observed in the guinea pig since it clears (erythema) sooner in the germfree rat (14 days) and hair grows back by 30 days. Conversely, our control conventional rats that were infected with T. mentagrophytes had scaly skin and no hair growth occurred at the inoculated site for 60-70 days.

The chronic T. mentagrophytes infection that persisted for 60-70 days in conventional rats is of interest because it was a low grade infection and not an ulcerating lesion like we saw in guinea pigs. This conventional rat model could be very useful for trials in topical or systemic therapy of fungal infections. The only visible evidence of dermatophyte infection was scaly skin and a lack of hair growth for 60-70 days. Fungi did penetrate and persist in the stratum corneum of the rat.

To date our studies have shown that skin from T. mentagrophytes lesion sites, in the conventional rat only, have a histopathological picture similar to psoriasis. This is a most interesting aspect of the work and it may indicate that a dermatophyte infection may be a mechanism for triggering psoriasis. Since it did not occur in the germfree state however, it may indicate that skin bacteria are involved in its etiology. These experiments on rats also demonstrate that conventional rats can carry T. mentagrophytes on their skin, without overt indications of dermatophyte infection, for prolonged time periods; culture and histology and no growth of hair were the only indications of a gross abnormality to the rats skin.

It should be remembered that in Vietnam, rats appeared to be an important vector for T. mentagrophytes. We have shown that rats have a capacity to carry T. mentagrophytes subclinically on the skin and in the GI tract for at least 70 days (termination of our experiment).

A significant stimulation of Peyer's patches was also observed in the small intestine of germfree rats infected with T. mentagrophytes. No fungi could be cultured or demonstrated with histological sections of Peyer's patches. There may have been some fungal products consumed by the rats that accounted for the stimulation of Peyer's patches.

Antibody and Cell-Mediated Immune Responses in the Dermatophyte Infected Germfree Rat:

AMI -- We have observed that germfree rats manifest a poor immunoglobulin response against the invading dermatophyte. A primary and secondary challenge of germfree rats with T. mentagrophytes resulted in an increased level of immunoglobulins in only one to six bacteria-free animals. Immunoelectrophoresis demonstrated no great increase in gammaglobulins within 70 days after challenge. We were only able to demonstrate one out of six rats showing a positive precipitin test to purified trichophytin, crude cell wall antigen and soluble cytoplasmic antigen. We assessed the capacity of serum from germfree, T. mentogrophytes monoassociated rats and conventional rats to inhibit T. mentagrophytes. Our results indicate that serum from the monoassociated rats is just as inhibitory as serum from germfree and conventional rats. Further work on these sera indicated that the inhibition of T. mentagrophytes by serum does not appear to be associated with specific immunoglobulin.

CMI -- In vitro blastogenesis of splenic lymphocytes against phytohemagglutin (PHA) and concanavalin A (ConA) are poor in the dermatophyte infected bacteria-free rats. However, the infected rats splenic lymphocytes appeared to acquire a good capacity (10-fold) to respond against the T. mentagrophytes antigens (crude autoclaved culture extract, formalinized spores, purified T. mentagrophytes antigen from Dr. Kerbs at LAIR). Lymphocyte blastogenesis (at various time periods after infection and clearing of the lesion) indicates to us that clearance of a dermatophyte infection appears to be associated with the acquisition of a CMI response in the rat.

It should also be pointed out that the skin testing of conventional rats that have the chronic infection gives a very positive delayed-type hypersensitivity reaction with purified T. mentagrophytes antigen, formalinized spores and crude autoclaved antigen. We did not see, however, a typical delayed-type hypersensitivity response in the monoassociated germfree rat. A delayed basophil response is very prominent in the conventional rat and this may indicate that a Jones-Mote type of reaction (cutaneous basophil hypersensitivity) is taking place rather than the typical pure monocyte response as seen in the classic delayed-type hypersensitivity response (to PPD) in tuberculin positive individuals.

Summary of Rat Experiments: This is a good model; a) the primary infection is not as severe as in guinea pigs. The erythema like reaction (with hyphae in skin) clears in the germfree rat in 13-14 days and the hair grows back. The hair does not grow back in conventional rats and a low grade persistent fungal reaction can be seen on the skin for 60-70 days. In conventional rats, there also appears to be a parakeratosis associated with the cleared infected site and it is very similar to psoriasis. The germfree rat does not become overgrown with the dermatophyte (T. mentagrophytes); only T. mentagrophytes caused any obvious dermatophyte-like pathology in the rat model. Epidermophyton floccosum induced a brown pigmentation (hyperkeratosis) over the inoculated site but no obvious fungal type of lesion. However, the latter pathology (hyperkeratosis) was observed in the skin of male but not female rats. Neither E. floccosum or M. canis survived in the germfree environment. They appeared to die out after each of several challenges of the germfree animal. This may indicate that bacterial associations are needed for the survival of the latter two agents on conventional rats.

Fungal Infections in Nude Mice: The nude mouse is an animal that is congenitally athymic; therefore, it lacks the capacity for the T-cell (CMI) arm of immunity. If dermatophyte infections are controlled by T-cell dependent immunity then the nude mouse should not be able to control dermatophyte infections. We were, however, not able to induce dermatophyte lesions on the skin of nude mice even though skin cultures were positive for T. mentagrophytes 7 days after challenge. Our procedure used a spore inoculum (100, 1,000, 10,000 spores per occluded site). A similar inoculum took very well on the conventional rat and the guinea pig. Thus either skin bacteria on a microbial flora activation of macrophages (i.e. with lipopolysaccharide or other B-cell mitogens) rendered the nude mouse resistant.

The nude mouse was further evaluated by IV injection of C. albicans. Our studies demonstrated that the nude mouse cleared an IV C. albicans challenge better than their littermates (i.e. same strain of mice but with a functional thymus). Candida albicans infections are thought to provide classic examples of the importance of T-cell function in host resistance to fungal disease. Our nude mouse data indicate that the importance of T-cell function in immunity to dermatophyte and candida infections is not as clear cut as one would gather from the existing literature. Our initial results on the nude mouse indicate host immune factors (other than CMI) could be operating to control fungal disease. We found the latter nudes (with proven T-cell function after thymic implants) were just as susceptible to an (IV) C. albican challenge as the normal mice. The implanted thymus appeared to suppress immunity to systemic candidiasis that was present in the athymic

nude mice. We are going to infect thymus implanted nudes to see if they can also now get a dermatophyte lesion on the skin. Direct macrophage activation might occur in conventional nude mice through lipopolysaccharide (from flora) stimulation or through contact with other B-cell mitogens.

Candida albicans Infections in Nude Mice and Tetracycline Treated Conventional BALB/c Mice and Germfree Mice. It is well acknowledged that Candida albicans infection of the alimentary tract frequently results from oral antibictic therapy or deficiencies in the cell-mediated immune (CMI) response (4). To further clarify the underlying mechanisms that initiate candidiasis (hyphal infection), we attempted to develop an animal model and a sensitive technique to assess hyphal infection of the alimentary tract.

Previous work (1,3,6) investigating the mechanisms by which oral antibiotic treatment enhances C. albicans infection, has not resolved whether the antibacterial, toxic or immunosuppressive effects of antibiotics are most responsible for candidiasis.

Since thymus-dependent immune deficiency, in humans, is also believed to predispose to oral C. albicans infection, the athymic mouse might serve as an experimental infection model. The animals congenitally lack a thymus which makes them deficient in the T-cell components of a normal immune response (5). Therefore, we felt that an oral challenge (C. albicans) in athymic and gnotobiotic mice might better explain whether a thymus-dependent immune deficiency, or the lack of microbial flora antagonistic to C. albicans is most important in predisposing to alimentary tract candidiasis.

MATERIALS AND METHODS

Organism: Candida albicans B311 Strain A was used in all experiments.

Animals: Male and female mice (BALB/c) were used in this study. Conventional mice were obtained from Charles River Breeding Laboratories, Inc. (North Wilmington, Mass.). The bacteria-free and flora-defined mice were obtained from the breeding stock at the University of Wisconsin Gnotobiotic Laboratory (Madison, Wisconsin). The conventional athymic (nude) mice were donated by Dr. Dean Manning of the University of Wisconsin (Department of Medical Microbiology).

Conventional mice were maintained in a standard animal room environment. Conventional nude mice were held in a 30°C incubator. The gnotobiotic mice were housed in flexible film isolators at the University of Wisconsin Gnotobiotic Laboratory and maintained by standard germfree techniques. Sterilized food (Ralston Purina 5010C) and water were consumed by all mice ad libitum. Suspended floor cages were used to minimize coprophagy.

Animal Inoculation: The yeast was grown in Sabouraud's Dextrose broth at room temperature for 4 days. The cells were then added to the drinking water at approximately 10^6 viable units (vu)/cc.

Normal drinking water was withheld for 6 hours (to stimulate drinking) prior to providing the mice with water containing C. albicans (yeast cells) for 18 hours. After this time, the yeast inoculum was replaced with normal water or tetracycline water.

Antibiotic: Tetracycline HCl (Sigma Chemical Co., St. Louis, Mo.) at a concentration of 1 mg/ml in distilled deionized water (pH = 2.5), was administered to treatment group animals in their drinking water. Control group animals

received acidified, distilled, deionized water. The antibiotic and control water was made available to the mice four days prior to the start of the C. albicans administration.

Sacrifice Protocol: Animals, randomly selected for autopsy, were asphyxiated with ether and the stomachs removed, placed immediately into glass vials, immersed in liquid nitrogen for at least 15 minutes as previously described (2) and stored at -70°C.

Total aerobic bacteria and C. albicans yeast counts were obtained from results of standard plate count dilution techniques of cecal contents.

Histology and Scanning Electron Microscopy (SEM): Stomachs were removed from storage at -70°C and cut still frozen, with a clean razor blade as follows: A cut was made from the ventral to the dorsal surface along the sagittal plane on the greater curvature. The resulting section showed the surfaces of the nonsecreting keratinized area, the glandular secreting area, and the mucosquamous junction of these two regions (cardial-atrium line). A second cut was made parallel to the first. This section showed a cross-section of the circumference of the stomach wall.

Histology: The cross-section of stomach was paraffin embedded, sectioned and stained with Periodic Acid Schiff stain (PAS).

SEM: The stomach section that showed surface glandular and keratinized areas was fixed and prepared for the scanning electron microscope through a modified procedure of that previously described (2).

Statistics: Student's t-test was used to evaluate the significance of quantitative differences of microbial counts in cecal contents. The significance level was chosen at $p < .05$.

RESULTS

In all experiments, total counts of C. albicans or aerobic bacteria in cecal contents did not differ significantly between males and females.

Conventional (Thymic) Mice. All animals sacrificed at each time period had quantifiable levels of aerobic bacteria in cecal material. The total aerobic bacterial counts, in the tetracycline treated group, were significantly lowered below those in controls (Table 1).

Not all conventional animals sacrificed at each time period had quantifiable levels of C. albicans in cecal material. Only 4/6 animals had detectable levels of yeast at the day 4 sacrifice, and yeast could not be detected in any of the remaining animals sacrificed after day 4.

In the antibiotic treated conventional mice, however, C. albicans counts also began to decrease after oral challenge, but eventually reached a stable level (Table 1). All tetracycline-treated animals sacrificed through day 4 had detectable levels of C. albicans (Table 1), but at day 16 and 32 only 2/6 and 5/12 animals had stable yeast levels.

Histology and scanning electron micrographs detected hyphal infections of the stomach in only 2/36 tetracycline treated animals: one male and female sacrificed on day 32 (Table 2). No control animals were infected. Yeast cells were not found adherent to the stomach surface in high quantities.

Conventional Athymic Mice The aerobic bacterial count of cecal contents from tetracycline treated animals was significantly lower than that of non-tetracycline treated controls (Table 1).

Candida albicans levels were slightly higher in the cecal contents of the tetracycline treated athymic mice, compared with controls, but the

difference was not significant (Table 1). Both tetracycline treated and control athymic mice, however, were colonized to higher levels than either treated or control conventional (thymic) mice (Table 1).

Tissue histology and electron microscopy did not bear out the prediction that athymic mice would succumb more easily to gastric candidiasis. Twenty-one of 24 animals did not show any gastric candidiasis, and only 3/24 animals developed hyphal infection of the stomach (Table 2). Candidiasis in the three athymic animals was not associated with tetracycline treatment.

The stomach glandular mucosa, in athymic mice, showed an increased colonization by yeast-phase C. albicans that was not commonly, or extensively seen in thymic conventional or bacteria-free mice. The yeast cells, without hyphae, also colonized the keratinized stomach surface, and at the cardial-atrium line, in 14/21 athymic animals.

Studies were also done on three flora defined athymic mice without tetracycline treatment. These animals contained a gut flora of nine bacterial species in the genera Lactobacillus, Bacillus, Clostridium, and Corynebacterium. The animals had significantly lower bacterial levels and significantly higher C. albicans levels at the five day sacrifice, than their conventional counterparts (Table 1).

In all flora-defined nude mice, oral yeast challenge resulted in infection of the cardial-atrium line and general keratinized stomach epithelium (Table 2).

Bacteria-free Mice. The quantitative levels of C. albicans in the cecal contents of bacteria-free mice, regardless of oral tetracycline treatment or time of sacrifice, was significantly higher than either treated or control, normal or athymic mice (Table 1). This higher level of yeast was reached in less than 48 hours, and was maintained at this level, without significant deflection, for the 32 day period.

Histological sections and electron micrographs detected mycelial infection of the keratinized stomach surface and cardial-atrium line in 8/10 animals sacrificed 48 hours after oral challenge (Table 2). Gastric candidiasis developed in 45/50 animals during the 32 day experimental period. The five animals without detectable C. albicans infection were distributed among the various sacrifice intervals (Table 2).

DISCUSSION

This study indicated that C. albicans colonization and infection in mice, is primarily modulated by the bacterial flora, which may be altered by oral tetracycline treatment or perhaps by T-cell immune deficiencies. Tetracycline, did not appear to enhance C. albicans infection either by growth stimulation, toxic effects or its converting the yeast to a more invasive form. Thus, an undisturbed bacterial flora may be more protective against gastric candidiasis than a competent immune response. Clinical application of these results might include control of the competitive normal flora to prevent high gut colonization levels by C. albicans in the compromised host.

This work also showed that the bacteria-free animal is a stable, predictable animal model for gastric C. albicans infection. Immunosuppressive drugs, antibiotic or irradiation treatment are not required for hyphae to invade gastric tissue. The immunodeficient athymic animal, both in the conventional or gnotobiotic state, also deserves further investigation to

elucidate mechanisms of fungal infection control through immune related flora alterations or specific immunity induced with passively transferred immune cells or serum.

The mouse stomach, especially the cardial-atrium line, was shown to be the most consistently infected site, and a reliable indicator of C. albicans infection.

Scanning electron microscopy was essential in accurately detecting total numbers of infected animals. Histological sections, while providing information on the host's cellular response against C. albicans, failed to detect almost 50% of all infections.

Table 1. Effect of oral tetracycline on the cecal aerobic bacteria and Candida albicans colonization in BALB/c mice.

Mouse Groups	Days after oral challenge with C. albicans: Tetracycline Treated — Bacteria 1-5	Bacteria 6-14	Bacteria 15-32	C. albicans 1-5	C. albicans 6-14	C. albicans 15-32	Non-tetracycline Treated — Bacteria 1-5	Bacteria 6-14	Bacteria 15-32	C. albicans 1-5	C. albicans 6-14	C. albicans 15-32
Thymus intact conventional	7.19±.11[a]	N.D.	6.51±.41	6.49±.20	N.D.	4.68±.48	9.37±.19	N.D.	8.77±.52	5.66±.28	N.D.	0.0
Conventional flora, athymic	6.61±.55	7.23±.17	6.86±.11	6.54±.27	6.93±.14	6.46±.14	10.08±.08	9.41±.39	9.95±.18	5.94±.23	6.30±.40	6.14±.07
Flora defined, athymic	N.D.[b]	N.D.	N.D.	N.D.	N.D.	N.D.	8.53±.14	N.D.	N.D.	7.46±.13	N.D.	N.D.
Gnotobiotic (bacteria-free)	N.D.	N.D.	N.D.	8.86±.06	8.63±.02	8.85±.05	N.D.	N.D.	N.D.	8.77±.07	8.63±.10	8.75±.03

[a]Mean ± standard error of $\log_{10}$ colony forming units per gram dry weight cecal contents

[b]Not done

Table 2. The influence of oral tetracycline on the infectivity of C. albicans in BALB/c mice.

Mouse Groups	Days after oral challenge with C. albicans					
	Tetracycline Treated			Non-tetracycline Treated		
	1-5	6-14	15-32	1-5	6-14	15-32
Thymus-intact, conventional	0/18[a]	N.D.	2/20	0/18	N.D.	0/20
Conventional flora, athymic	1/4	0/4	1/4	1/4	0/4	0/4
Flora-defined, athymic	N.D.[b]	N.D.	N.D.	3/3	N.D.	N.D.
Gnotobiotic (bacteria-free)	9/10	5/5	9/10	7/10	5/5	10/10

[a]Total number of infected animals/total number of animals sacrificed within each time period.

[b]Not done

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